

JUL 21 1908

MATERIALS FOR A MONOGRAPH OF THE ASCONS. 301

Materials for a Monograph of the Ascons.

II.—The Formation of Spicules in the Genus *Leucosolenia*, with some Notes on the Histology of the Sponges.

By

E. A. Minchin, M.A.,

Professor of Protozoology in the University of London.

With Plates 17—21, and 5 Text-figures.

CONTENTS.

	PAGE
I. Introductory	301
II. The Monaxon Spicules	305
III. The Triradiate Systems	315
IV. The Gastral Rays	319
V. Derelict Spicules in <i>Leucosolenia complicata</i>	321
VI. Histological Notes; the Excretory Cells of <i>Leucosolenia complicata</i>	322
VII. An Abnormal Triradiate	327
VIII. Some Observations on the Disposition of the Triradiate Systems of <i>Leucosolenia lieberkühni</i>	327
IX. General Remarks on the Formation of Calcareous Spicules	334
Bibliography	347
Description of the Plates	349

I. INTRODUCTORY.

In former memoirs (1896, 1898, 1905 [1]) I have proposed to divide the Ascons, or Calcarea Homocœla, into two families, Clathrinidæ and Leucosoleniidæ, differing

from one another in all those characters which, in my opinion, are of fundamental importance for the classification of Calcareous Sponges. I believe that the two families in question represent the most deeply-rooted phylogenetic divergence in the Calcareous Homocœla, and are two natural systematic groups, one of which—namely, the Leucosoleniidae—approaches far nearer to the ordinary Heterocœla, such as *Sycon*, *Grantia*, *Leucandra*, etc., than does the other. The genus *Leucosolenia*, Bowerbank, in the sense in which the name is employed by me (1905 [2]), constitutes the principal, if not the only, genus in the family Leucosoleniidae, while the genus *Clathrina* occupies a similar position in the Clathrinidae.

Having in a former memoir (1898) described the origin and growth of the triradiate and quadriradiate spicules in various species of *Clathrina*, it seemed to me important to supplement the results then obtained by a study of spicule-formation in *Leucosolenia*. The observations put forward here have been carried on, at rather long intervals and with many interruptions, over several years, and some of my figures are now more than eight years old. I may be allowed to mention this in order to explain the relation of my investigations on calcareous sponge-spicules to those already published by my friend and former pupil, Mr. Woodland. After I had observed the principal facts in the development of the spicules of Leucosoleniidae I suggested to Mr. Woodland that he should study the spicule-formation in the Heterocœla; he proceeded to do so in *Sycon*, and found a mode of origin for the spicules in this sponge perfectly similar to what I had found in *Leucosolenia*. I had intended to publish my observations a long time ago, but various obstacles and pre-occupations prevented my doing so, and in the meantime Mr. Woodland's studies have been published (1905). It is in no way with the view of detracting from the value of Mr. Woodland's researches, which he has extended over a wide field, but simply in order to protect myself against a possible charge of plagiarising from his results that I point out here (as Mr.

Woodland has also done) that my figures and investigations on calcareous spicules antedate his, though published later.¹

In my former memoir (1898) I went fully into the literature of spicule-formation in sponges and discussed the various statements of fact or theory made by those who have written upon this subject.² I may refrain from discussing further the historical side of the subject, since most of the papers dealing with the spicules of sponges that have been published during the past few years will be found quoted by me, with brief abstracts, in the 'Zoological Record' (1900—1903). I shall therefore only refer to the statements of other authors as occasion arises. In one point the published statements upon spicule-formation led me astray in my former memoir. Having then had no opportunity of investigating primary monaxons in the Clathrinidæ, I took for granted the correctness of previous statements upon the formation of monaxon spicules in sponges with regard to one point, namely, that each such spicule developed and grew in a single cell. This alleged mode of development was an obstacle to my comparison of the triradiate spicule to a system of three monaxons joined together, since each ray of a triradiate is formed from the first by two cells. When I came to investigate the monaxons of *Leucosolenia*, however, I found that each spicule of this class, whatever its size or form, arose invariably in two cells, exactly as does a single ray of a triradiate system. This fact had previously been observed by Bidder (1898, p. 62, foot-note) in the case of the hair-like spicules of *Grantia compressa*, and was regarded by him as specially characteristic of this type of monaxon; but from the observations of Woodland and myself, it would appear to be uni-

¹ I take this opportunity of remarking that I regard Woodland's "Studies in Spicule Formation" as constituting a most valuable contribution to our knowledge, so far as matters of fact and observation are concerned; but that in matters of theory I am often unable to see eye-to-eye with him. I may be obliged in the course of this memoir to controvert his speculative opinions.

² Maas (1900 [3], p. 555) is very charitable to me in attributing to design, and not to inadvertence, the fact that in my memoir (1898) I overlooked the statements of Deszö on spicule-formation in *Tethya*.

versally true, at least for the primary monaxons of *Calcarea*, that each such spicule is produced by two formative cells. This removes the only difficulty in the way of deriving the triradiate systems from a fusion of originally separate monaxons.

The species of *Leucosolenia* studied by me are *L. complicata* and *L. variabilis*. I have elsewhere (1905 [2]) given a detailed description of the characters, and especially of the spiculation of these two species. The material was obtained from Plymouth, and I have to thank the authorities of the Marine Biological Association for the trouble they have taken in collecting and preserving these sponges according to my directions. The technique used by me was the same as in the case of *Clathrina*, namely, preservation fresh from the sea in $\frac{1}{2}$ per cent. osmic acid, staining with picocarmine (either Ranvier's or Weigert's picocarmine, supplied by Grüber and Co.), and examination in neutral glycerine or Canada balsam. This method is excellent for all protoplasmic structures, and does not corrode the spicules in the least; but it does not show details of nuclear structure, though the nuclei as a whole are well shown and clearly differentiated from the cell-body. For nuclear structures acid fixatives are indispensable, but then, of course, spicular structures are destroyed.

I have studied the stages of spicule-formation principally in surface-views of the thin wall of the sponge laid out flat, and rely upon sections only for confirming and supplementing the results so obtained. But I have abandoned the method which I formerly practised of brushing the collar-cells away with a paint-brush, as I now believe this procedure to be a dangerous source of error. The young stages of the spicules are found so close to the gastral epithelium (see especially Pl. 18, figs. 50—52, 54, 55, etc.) that I am now convinced that brushing may remove spicule-forming cells as well as collar-cells, and that some of my former figures (1898, Pl. 38, figs. 9, 12) are, in consequence, erroneous and incomplete, as I shall have occasion to point out further below. I

now rely on the region of the oscular rim, where the collar-cells are entirely wanting. By carefully cutting off the sponge-wall in this region and laying it out flat without any further treatment, stages of spicule-formation can be found and drawn, and thus any possible error arising from manipulation is entirely eliminated.

In *Leucosolenia* the dermal epithelium covering both aspects of the oscular rim is comparatively free from the coarse granulations found in *Clathrina*, a fact which makes the former genus a particularly favourable object for demonstrating the stages of spicule-formation, even to classes of students. It is interesting to find that in these days of advanced technique, there are still histological facts to be discovered by methods which might have been feasible to Lieberkühn or Max Schulze.

The investigations set forth in the following pages are not quite so complete in some points as I could have wished. Since, however, I now hold an appointment in which it is accounted to me for unrighteousness to look at a sponge (except for domestic purposes), I am obliged to put forward my results as they are without spending time upon further investigations.

II. THE MONAXON SPICULES.

The formation of the monaxon spicules of *Leucosolenia* takes place in a manner essentially uniform, in all cases observed by me, whatever the shape or size of the spicule. The process may therefore be described in general terms, and the variations observed in particular cases will be briefly noted afterwards. As a preliminary it should be pointed out that the full-grown monaxon spicules of *Leucosolenia* (the statement can, perhaps, be extended to the primary monaxons of all *Calcarea*) are placed invariably with one end, which may be called proximal, imbedded in the body-wall, while the opposite, or distal extremity, projects freely into the water. Haeckel (1872, vol. i) states that the

monaxons have their long axis parallel to the canal, in the wall of which they lie (l. c., p. 297), and are placed "in a meridian of the direction of the current" (l. c., p. 298). A glance at any *Leucosolenia* is sufficient to dispel this illusion; in all parts of the sponge the monaxons are found pointing in every possible direction (compare Fig. 108, Pl. 21); only at the oscular rim is a slight tendency to regularity noticeable.

The first sign of the formation of a monaxon spicule is the division of the nucleus of a dermal epithelial cell, followed by incomplete division of the cell-body (fig. 1—3, 41, 73—75). This division of the mother-cell, as it may be termed, marks out the two formative cells, which are not, as a rule, completely separated from one another in early stages, but later become distinct and largely independent of one another. A further point to note is that the formative cells retain a connection with the dermal epithelium from which they are derived (figs. 43—48; especially fig. 45). This point is of importance, since Woodland (1907) has based theoretical deductions upon the assumption that the scleroblasts have "lost connection with the rest of the organism" (l. c., p. 60). He even speaks of the scleroblasts as "wandering cells," meaning thereby, as I understand him, not that they belong to the class of amœbocytes (i. e. archæocytes), but that they are completely independent of other histocytes. It is always more easy to overlook than to discover connections between cells, and, therefore, positive observations are of more value than negative ones in their bearing on questions of this kind; but, in any case, I should doubt if scleroblasts, or skeletal tissue generally, could be supposed to be more independent than any other class of tissue-cells, and I consider the term wandering cells misleading as applied to the scleroblasts, and likely to produce a mistaken theoretical bias.¹

¹ Woodland admits a connection between the scleroblasts and the dermal epithelium in some cases (1905, p. 243, and Pl. 15, figs. 45—49), but considers that in other cases no such connection is retained (l. c., fig. 48).

Each of the two formative cells plays a peculiar and characteristic rôle in the production of the spicule; one places itself more proximally, that is to say, deeper in the body-wall, and migrates towards the gastral surface; the other remains in a more distal, that is to say, superficial position, and is found in, or near to, the dermal epithelium. The proximal cell is conspicuous as a rule, for its more irregular form and amœboid appearance. As it travels inwards it forms the shaft of the spicule, and its chief function appears to be to determine the length and direction or curvature of the spicule. Like the apical formative cells of the rays of the triradiates, its influence is apparently chiefly directive; hence it will be briefly referred to in the sequel as the founder. On the other hand, the more superficially placed formative cell is more compact as a rule, and is concerned chiefly with building up the spicule to its full thickness. Like the basal formative cells of the triradiates, its function is essentially secretive; hence it will be termed for short the thickener.

Founder and thickener are at first scarcely distinct from one another, and the first sign of the spicule is seen between the two nuclei, but chiefly in the sphere of influence of the thickener (figs. 3—5, 9, 42, 45, 48, 76, 77). The earliest part of the spicule to appear is the distal extremity, the end,

A further point which Woodland has raised, and upon which it is more difficult to obtain decisive evidence, is whether or not the monaxons have in all cases a single mother-cell. He is of opinion that in some cases two distinct epithelial cells co-operate, and furnish the two formative cells. His observations could, however, be interpreted as meaning simply that in some cases the division of the primary mother-cell is complete, in other cases incomplete; and since the two cells become perfectly distinct later on, as Woodland himself has shown, the former procedure is more likely to be the primitive one. For my part I am inclined to regard the monaxons as uniform in origin and to refer them in all cases to a single mother-cell, but from the nature of the case it is almost impossible to give a decisive judgment on this point; nor do I consider it a fair argument to attempt to prejudice the point, as one might be tempted to do, by the analogy of the formation of the rays of the triradiates.

that is to say, which ultimately projects freely from the sponge-wall into the surrounding water. In most cases the free extremity of a monaxon spicule is marked by the possession of a so-called lance-head, a characteristic feature which is seen, when carefully examined, to be in reality a double bend in the shaft of the spicule, comparable rather to a bayonet than to a spear-head. The thickener remains attached to the head until this part has attained its full thickness and dimensions, but the founder travels away from the head, and lays the foundation of the shaft as a slender rod, which ends at first in the sharpest of points, and is far thinner than it becomes later (figs. 5, 6, 10—12, 43, 78). When the head of the spicule is fully formed, the thickener migrates slowly along the shaft towards the proximal extremity to which the founder is attached, and as it travels down it builds up the shaft to its full, definitive thickness (figs. 7, 13—16, 79—81). No further increase in size is possible for that part of the spicule beyond which the thickener has passed in its course towards the proximal end. Thus the function of the thickener is to finish what the founder has begun. In many cases the activity of the thickener is indicated by a distinct rim, marking off the distal portion of the shaft, formed to its full thickness, from the proximal slender portion laid down by the founder alone (figs. 13, 14 *r*, 79, 80, 87). As the distal extremity of the spicule is completely formed it begins to project from the dermal surface of the sponge, being pushed outwards from the body-wall. How this extrusion of the spicule is effected is not quite clear, but it is perhaps due to the secretive activity of the founder-cell. The migration inwards (i. e. gastral-wards) of the founder in the early stages of the growth of the spicule soon brings it in contact with the bases of the collar-cells (see figs. 43—45, 71*a*, 71*b*). Beyond this point it cannot go, and the subsequent growth in length of the spicule is not really due to any further migration of the founder in a proximal direction, but to a pushing out of the spicule in a distal direction. Though the founder and thickener appear

to migrate along the shaft in a proximal direction, they are, in reality, practically stationary, while the spicule moves outwards. Sections show that the thickener retains its connection with the dermal epithelium, and forms a constituent cell of this layer (figs. 42, 43, 45—48, 57). Very noteworthy is the frequent presence in the founder-cell of a distinct, clear space in the protoplasmic body, continuing the shaft of the spicule, and appearing like a mould in which the secretion of the growing shaft is laid down (figs. 5—7, 13, 15, 79—83, 85, 86). When the preparation has been very slightly corroded by the mounting medium or some other cause, the proximal end of the shaft often appears hollow, containing a cavity or canal continuous with the clear space in the protoplasm of the founder-cell (figs. 83—86). This appearance, perhaps, indicates that the first secretion produced is of organic material which becomes subsequently impregnated with calcite to form the spicules, an interpretation further borne out by the observation that in some cases, in preparations in which there was not the slightest evidence of corrosion, the very young spicule appears first as a clear space within the protoplasm of the two formative cells, and that the calcite, when it makes its appearance, does not at once completely fill this space (figs. 3—5). In this connection I may draw attention to the very interesting results of Maas (1904 [1], [2], 1906), who has found that sponges reared in water deprived of CaCO_3 produce organic sclerites, not containing calcite.

When the spicule is nearly fully formed the founder ceases its activity. As the thickener, however, continues to secrete and to pass towards the proximal end of the shaft, the two formative cells are brought close together (figs. 7, 8, 17, 18, 44, 57, 71A, 82, 83, 85—88). When the spicule is fully formed, both cells leave it altogether. According to Woodland (1905, p. 242) the founder leaves it first (compare my fig. 8) and the thickener later; a fact which proves clearly the complete distinctness of the two formative cells in the later, if not in the earlier, stages of spicule-formation. The

thickener rounds off the proximal end to some extent and completes it. Full-grown spicules can always be distinguished from those not quite completed by the condition of the proximal end (compare figs. 87, 88, and 89). With a little practice the eye picks out the unfinished spicules rapidly in the preparation, and more careful inspection reveals at once the two formative cells on the shaft. The abandonment of the spicule by the formative cells is practically the only point in which the development of the monaxon spicules differs from the rays of the triradiates, on which one cell, at least, namely the thickener, is retained.¹ The difference is perhaps related to the fact that the triradiate systems are entirely imbedded² in the body, while one end of the monaxons always projects freely. The monaxons are probably being continually shed, or, it may be, drawn out of the sponge, like the hairs of many caterpillars. Hence the cells leave them, and new monaxons are continually being formed. The triradiate systems, on the other hand, are more permanent skeletal elements, their chief function being support, and not defence or protection, and in consequence they retain their formative cells.

I pass on now to describe briefly some of the peculiarities of the formation of monaxon spicules in particular cases.

(1) *Leucosolenia complicata*. In this sponge there are two sharply distinct types of monaxon spicules (*a*) small, straight, slender forms, and (*b*) large, curved, stout forms. No transitions are found between these two types (Minchin, 1905 [2]).

(*a*) The slender monaxons are very abundant, as a rule, and their early stages are easily found, especially in the oscular rim. Sections show that in this region they may be

¹ Compare p. 319 below.

² Woodland (1905, p. 237) states that in many Ascons the triradiates protrude through the thin body-wall, "so resembling monaxons." I can only say that no such Ascons are known to me. I may point out further that the development shows clearly that the apices of the rays of the triradiates are comparable to the proximal ends of the monaxons, not to the distal projecting ends.

formed in the interior of the sponge wall, projecting into the gastral cavity (figs. 46, 57, 68), in which case they arise from the dermal epithelium lining the gastral wall of the oscular rim in exactly the same way as they are formed on the outer surface of the sponge. The fact that the inturned dermal epithelium, which is for the most part destined to give rise to porocytes, can also secrete monaxons, is an important point in considering the origin of the gastral rays of the quadriradiates.

The slender monaxons of *L. complicata* are remarkable in that the lance-head is either rudimentary (fig. 7) or absent (fig. 8). The stages in the formation of these spicules are shown in surface views in figs. 1—8, and in sections in figs. 42—47 and 57.

The most noteworthy points in this development are, first, the great distance between the two formative cells before the spicule appears; and secondly, the clear space, which has been described above, which precedes the formation of the spicule (figs. 1—5). In places where the body-wall is thickened, for instance, at or near the point of attachment of the sponge, the spicules may be formed very far in from the surface (fig. 45).

(b) The stout monaxons are fairly abundant, but their earliest stages are, for some reason, difficult to find; perhaps because they grow with great rapidity. As a rule they have a very distinct lance-head, and figs. 10—15 show, without need of further explanation, how the lance-head is formed by the thickener and then gradually abandoned by it. Occasionally, however, the lance-head is completely absent (fig. 16). The great size of these spicules makes it very difficult, in fact, practically impossible, to obtain perfect stages of their growth in sections. Some are shown in figs. 48, 71 *a*, 71 *b*.

When I commenced my investigations on these spicules I made use of the method, which I now condemn, of brushing off the gastral collared epithelium. In such preparations I found many young spicules, similar to figs. 10 and 11, but

with only one cell on the spicule, this cell being situated on the lance-head in the position of the thickener. At first I regarded this state of things as normal, but I am now convinced that it arose simply by the brush having removed the founder, which is often situated very close to, or even among, the collar-cells. (Compare Figs. 48 and 71 *b*.)

(2) *Leucosolenia variabilis*. In this sponge the ordinary monaxons vary greatly in size but agree in their characters, and form one class in which every gradation can be found between the two extremes of size. They have distinct lance-heads and long, curved shafts. Two other types of monaxons are also found, but they are very scarce, and I have not found any stages of their development.

The ordinary monaxons may conveniently be grouped into small (fig. 83), medium (fig. 86), and large (fig. 89), but these three sizes are connected by every possible gradation, and must not be regarded as indicating distinct subdivisions. There is nothing to note about their mode of development which has not been said already, but some conclusions of importance from a systematic point of view may be drawn from the stages of growth. It is sufficiently obvious from the figures that small monaxons like fig. 83 are by no means to be regarded as young stages of large monaxons like figs. 88, 89. On the contrary the future size of the spicule is determined at a very early stage—that is to say, as soon as the lance-head is formed (compare figs. 78 and 84). Further, the study of the development affords a means of distinguishing the spicules that have or have not attained their full growth, as already pointed out. In describing the spiculation of a sponge for purposes of specific identification it is most important that the description should apply only to fully-formed spicules, and not to those in which the growth is not complete.

The mode of development of the monaxon spicules that I have described in the foregoing is one that I have found in all the monaxons of *Leucosolenia* that I have studied, and Woodland, whose preparations I have looked through, has

found a similar course of events in the formation of the monaxons of *Sycon*. Maas, however, has given a description of the formation of the monaxons of *Sycandra setosa*, which is at variance with our results (1900 [2]). He finds the small monaxons developing each in a single cell, but the larger monaxons, on the other hand, being built up by numerous cells. It may be pointed out, to begin with, that Maas has described these appearances from sections, which is an unsafe proceeding. I should interpret his fig. 23 on Pl. 11, as representing one of the two cells present, probably the thickener, on each of the three spicules depicted, the other cell having been cut away or overlooked. Again, his fig. 25, intended to represent a large monaxon with many cells on it, is not convincing to me, as it is not possible with the magnification given (300) to determine the relation of the cells to the spicule. The drawing shows six rounded cells on or over the spicule, none of which look at all like scleroblasts. I may remark that the monaxon in question is very much smaller than the large monaxons of *L. complicata* (compare my figures of the spicules at the same magnification [1905 (2)]); and that in this case, as already described, I have found never more than two cells present on the spicule. I regret, therefore, that I am unable to accept my friend's figures as evidence for the correctness of his statements, which appear to me to require revision.

A monaxon spicule which develops, like a single ray of a triradiate, from two formative cells derived by division of a single mother-cell, is a spicule which I should regard as a primary monaxon. So far as the observations of Woodland and myself extend, the monaxon spicules of *Leucosolenia* and *Sycon* are all primary. There is no reason why a monaxon spicule should not arise secondarily, by modification of a triradiate, that is to say, by loss of one ray and placing the two remaining in a line with the other. I have elsewhere (1905 [3]) given reasons for regarding the huge monaxons of *Clathrina contorta* as secondary in nature, and I feel convinced that the same applies to the elbowed monaxons in

the stalk of *C. lacunosa*, and probably to many other cases also. In all the species of Clathrinidæ in which I have been able to follow the development, the first spicules formed after the metamorphosis are triradiates, and monaxons, if present, appear much later. On this account I am inclined to doubt if primary monaxons occur at all in Clathrinidæ. In the few cases in which I have found developing monaxons in species of Clathrina, there were several formative cells attached to them, at least four, and not two only, as in *Leucosolenia*. I had greatly hoped to have been able to study the development of monaxon spicules in Clathrinidæ, the only thing required to complete our knowledge of the formation of spicules in Homocœla. I must, however, leave this task to others, and can only say at present that there is some evidence for believing the monaxons of Clathrinidæ to be of secondary nature. I may add that there is no reason why secondary monaxons should not occur in *Leucosoleniidæ*, but there is at present no evidence that they do. Maas's statements raise a presumption in favour of the occurrence of secondary monaxons in *Sycons*, but his single observation does not convince me on this point.¹

According to Maas (1900 [1], p. 44; [2] p. 225) the small monaxons of *Sycandra setosa* grow at first slowly within a cell, until they project from the cell and from the tissue, and then a sudden, rapid growth of the spicule takes place, like the shooting out of a crystal ("Krystallartiges Anschliessen"). If I have understood the author's meaning rightly, he considers the portion of the spicule imbedded in the tissue as the older part, and the portion projecting into the water as a more recent formation. My observations lead, however, to an exactly opposite conclusion. The development of monaxon spicules possessing a lance-head shows clearly that the distal extremity of the spicule is the part first formed, and the proximal portion is the last to be secreted by the scleroblast.

¹ I consider it possible that the bayonet-like monaxons described by me (1905 [2]) in *Leucosolenia variabilis* (l. c., text-figs. 95, 16 *g*, 17 *a*, *b*) and *L. botryoides* (l. c., text-figs. 98, 25 *i*, *j*) might be reduced triradiates.

III. THE TRIRADIATE SYSTEMS.

One of the distinctive characters of the genus *Leucosolenia*, as defined by me in a former memoir (1905 [2]), is seen in the form of the triradiate systems. In *Clathrina* these spicules are typically equiangular, and only very exceptionally depart from this type, equiangular triradiates being in all cases present and constituting the principal skeleton. In *Leucosolenia*, on the other hand, the triradiates exhibit typically a bilaterally symmetrical pattern; they have a unpaired straight ray and two paired, usually curved rays, and corresponding to these differences in the rays, there is an unpaired angle greater than 120° , and two paired angles each less than 120° . Only exceptionally, and as one of many variations, are the three rays equally developed, or the three angles each indistinguishable from 120° . The two species in which I have studied the spicule-formation are further distinguished from one another by the characters of their triradiates. In *Leucosolenia complicata* the unpaired ray of the triradiate is almost invariably longer than the paired rays; in *L. variabilis* the unpaired ray is constantly the shorter of the three. Moreover, in *L. variabilis* the unpaired angle is much more obtuse than in *L. complicata*, and often approaches 180° , while in *L. complicata* it more nearly approximates to 120° .

If an ordinary museum-specimen, preserved in spirit, of *L. complicata* be taken, and a piece of the thin body-wall cut out, mounted without further treatment, and examined microscopically, with moderately high magnification, it is easy to see many stages of the growth of the triradiates, especially if the preparation be examined by polarized light with crossed prisms, when the spicules stand out brilliantly illuminated on a dark background. It is then seen that the stages of growth are as follows:—first a small piece of calcite in the form of an isosceles triangle, in which the base is about one-third the length of the sides; this represents the

unpaired ray. Next, from the base of the triangle two tiny rays grow out, which become larger and take shape as the two lateral rays of the spicule, growing rapidly, and so reducing the disproportion between the paired and unpaired rays, which is at first so marked. From the comparison of a series of young stages in the growth of the spicules the impression gained is that the triradiate system is laid down first as a monaxon spicule representing the unpaired ray, and that this monaxon spicule branches at one end, thus giving rise to the two paired rays. This interpretation of the observations seemed to me so obvious that, until I had studied the behaviour of the formative cells, I regarded the spicules of *Leucosolenia* as arising by secondary branching of a monaxon, and, therefore, of a fundamentally different type from those of *Clathrina*, which represent a system of three monaxons joined together. As soon as I had studied the histological details of spicule-formation, however, I found this interpretation to be quite erroneous, since the triradiate systems of *Leucosolenia* develop in just the same way as those of *Clathrina*, namely, from a sextett of cells, two of which give rise to each ray of the spicule. The rapid growth of the unpaired ray of *L. complicata* is merely an interesting case of precocious formation which is prophetic, so to speak, of the great size to which this ray ultimately reaches (figs. 21—29). If one examines the development of the triradiate systems of *L. variabilis*, in which the unpaired ray is the shortest of the three, it is found at once that there is no such precocious development of any one of the three rays.¹

The "sextetts" of formative cells from which the spicules arise are found without difficulty in surface views or sections (figs. 19, 20, 51, 54, 55, 71, 90, 91). Though I do not doubt that each sextett arises, as in *Clathrina*, by division of each cell of a "trio," it is more difficult to be sure of this in *Leucosolenia*. I have not found any distinct trios, as I did in *Clathrina*, and, except for the argument by analogy

¹ Woodland has recorded analogous differences in the development of the triradiates of *Sycon ciliatum* and *S. coronatum* (1905, p. 245).

furnished by the development of the monaxons, it would be quite feasible to regard each sextett as having arisen by the immigration of six cells independently from the dermal layer. The cells of the sextetts are very irregularly disposed, and it is difficult, often impossible, to pair them off, so to speak. Frequently a seventh cell, slightly more granular than the remaining six, is found, forming a septett; in these cases the spicule is destined to be a quadriradiate, and the seventh cell is a gastral actinoblast, the mother-cell of the gastral ray. I have never observed, however, the gastral actinoblast to be present until the triradiate system has begun to be formed and has reached a certain size.

Although I have examined carefully some hundreds of young stages in the formation of calcareous triradiate systems, both in my preparations and in those of Woodland, I have never seen anything but the two conditions I have just described, that is to say, either six or seven cells. Urban (1905, Pl. 6, fig. 34) has figured, however, a triradiate system with more than six cells upon it. I am inclined to think that this author has not clearly distinguished between formative cells adherent to the spicule, and cells of the overlying dermal epithelium. It is very easy to confuse the two. More difficult for me to understand are the statements of Maas (1900 [2]), who claims to have found the quadriradiates of *Sycandra setosa* arising each in a single cell (l. c., Pl. 11, fig. 24, i—iii). This statement is so directly at variance with all my experience on the subject that I am unable to support my friend's statements. I think the discrepancy in our observations may be explained in the following way. In the septett of cells covering a young quadriradiate the gastral actinoblast is far more granular than the formative cells of the basal system, and this difference is accentuated if alcohol is used as the preservative (which was Maas's method) instead of osmic acid (which I always employ). Looking down upon the septett from the gastral aspect, the gastral actinoblast would be obvious, but the cells of the underlying sextett might easily be overlooked if

they were faintly stained. One would then get the impression that the entire spicule was the product of one cell, namely the gastral actinoblast. I may point out that so careful an observer as Metschnikoff made, many years ago, a similar misinterpretation of the sextett surrounding the young triradiates.

It is never safe to be dogmatic about things one has not seen, but I venture, nevertheless, to express my belief that it will be found to be universally true that the triradiates of calcareous sponges develop from six cells,¹ the quadriradiates from seven.

As regards the subsequent development of the triradiated systems, the process of events is quite similar to what I formerly described in *Clathrina*, as may be seen from my illustrations. The first deposits of calcareous matter are very irregular (figs. 19—21), but soon take definite shape as a symmetrical spicule, and, concomitantly, the cells of the sextett sort themselves out into three pairs of formative cells, two attached to each ray in a definite and uniform manner. One formative cell, placed more towards the gastral aspect of the body-wall, behaves in all respects like the "founder" of the monaxon spicule; the other, placed more on the dermal side, is the "thickener" (figs. 24—28, 52, 58, 92—95). The founder is to be sought for at the tip of the ray, the thickener at the base; hence these two cells were distinguished by me in a former memoir as the apical and basal formative cells, respectively. As development proceeds, there comes a period when the founder is no longer to be observed, having wandered off from the ray. It is with regard to this point that I wish to correct some of my former statements. In *Clathrina* I figured a quite young spicule, in which the rays were far short of their full length, without any founder-cells attached to the extremities of two of the rays (1898, pl. 38, fig. 9). I am now convinced that this condition was simply due to the founders having been brushed off, since they are

¹ That is to say, in the first instance from three mother-cells, each of which divides into two, thus giving rise to the sextett.

placed very close to the gastral epithelium (figs. 50, 52). Examination of preparations not manipulated in any way shows that the founder, as in the case of the monaxons, does not leave the ray until it has attained its full length (figs. 29, 97, 99). In *C. clathrus*, as I showed formerly, the founder does not leave the ray at all and is thus responsible for the clubbed ends of the rays which are distinctive of this species.

As soon as the ray has grown to its full length, however, the founder disappears. The thickener has, meanwhile, migrated slowly outwards from the base of the ray, depositing lime and finishing off the ray as it passes along. Shortly before the disappearance of the founder, the thickener is found close to it just as in the monaxon spicules (compare figs. 97 and 99 with figs. 7, 8, 17, 18, 57, 71a, 82, 83, 85—88). In the fully-formed triradiate each ray bears the thickener at the extreme tip, generally in the form of a compact cell, but sometimes remarkable for sending out slender processes in various directions (figs. 97, 98). The thickener does not quit the ray, but can always be found attached to the tip of it. Woodland, however, states (1905, p. 145) that in *Sycons* both formative cells quit the rays of the triradiates. I can only say that in all the *Ascons* I have examined, I have never failed to find at least one cell on the apex of each ray of the full-grown triradiate systems.

IV. THE GASTRAL RAYS.

In the genus *Lencosolenia* the gastral rays of the quadri-radiates are always curved and thorn-like, the apex pointing, typically, towards the osculum, i. e., in the direction of the water-current.

In the formation of the gastral rays, I found a curious difference between those formed in the oscular rim, above the level reached by the gastral epithelium of collar-cells, and those formed throughout the gastral cavity generally where it is lined by collar-cells. I will deal with the latter first. As

I have already stated, the gastral ray owes its origin to a granular cell which joins itself to the six formative cells of the triradiate system to form a septett. In *Clathrina* I was able to show that this cell, the gastral actinoblast, arose from a porocyte, or from one of the epithelial cells, lining the interior of the gastral rim, from which porocytes arise. In *Leucosolenia* the characters of the gastral actinoblasts leave no doubt in my mind that they have an origin similar to those of *Clathrina*, but I am not able to bring forward such definite proof of this statement. This is one of the points in which I had hoped to have completed my observations. I may draw attention, however, to the section figured in fig. 54, in which the cell (*p. c.*) could be interpreted as a gastral actinoblast in the act of migration from the neighbouring pore.

In *L. complicata* the nucleus of the gastral actinoblast divides sooner or later into two. Sometimes the division takes place very early (fig. 24).

There is little to note with regard to the growth of the gastral ray itself; it is sufficient to refer to the illustrations given (figs. 24—28, 60—70). I may draw attention, however, to the fact that the gastral ray of *L. complicata* arises far behind the junction of the three basal rays (figs. 24—26, 28, 52). This point is noticeable in the full-grown spicule, but much more so in the early stages of growth. Hence even quadriradiate spicules, at their first appearance, are utterly unlike the primitive tetraxon form, from which some authors derive all the spicules of calcareous sponges.

With regard to the cells found upon the gastral ray some remarkable peculiarities can be observed. One is the tendency of the cells to become increasingly granular, some of the granules being of large size and taking a pink stain with picrocarmine. Another point is the presence in many cases of additional cells of a peculiar kind on the full-grown gastral ray (figs. 64—67, *ex. c.*, 69); a point which I shall have to deal with under a separate heading.

As regards the gastral rays formed in the region of the

oscular rim above the limit of the collared gastral epithelium, I was unable to find actinoblasts upon them in sections, and it appeared to me as if the gastral rays in this region were formed directly by the activity of the dermal epithelium lining the interior of the oscular rim (figs. 56, 68). My observations, however, are not sufficiently extensive to prove this point to my satisfaction.

V. DERELICT SPICULES IN *LEUCOSOLENIA COMPLICATA*.

In *L. complicata* I found frequently abnormal spicules which were evidently deformed triradiates or quadriradiates, and which were situated close to the dermal surface (figs. 30—36). Some had no cells attached to them, others had one or more cells, in one case (fig. 36) as many as four, but never the full number of six or seven found on normal spicules.

These peculiar bodies appear to me to be spicules which for some reason have become arrested in their development and abandoned by their formative cells at an early stage in their growth, and are about to be extruded from the sponge. In one case the appearance seen (fig. 35) suggests that the formative cells were in the act of migrating from the spicule. The peculiar forms of these spicules appear to be due to the fact that one or more of the formative cells may adhere to the spicule for some time, and continue to secrete calcite. Figs. 30 and 34 suggest this interpretation strongly. When all the cells have left them they become mere derelicts, which react as foreign bodies and are cast off.

If my interpretation of these peculiar bodies is correct, they afford a certain analogy with the formation of the large monaxons of certain *Clathrinidæ*. As I have stated above, I found four formative cells on the monaxons of an undetermined species of *Clathrina*; this observation, by comparison with the primary monaxons, indicates that the big, secondary monaxons of *Clathrinidæ* are biradiates, that is to say, are equivalent each to two rays of a triradiate, a view which I have maintained elsewhere (1905[3]) for those of

Clathrina contorta and *C. lacunosa*. I believe, however, that in an earlier stage the secondary monaxons would be found to be covered by six cells, of which two wander off, leaving the two remaining pairs to secrete the spicule. On this view the secondary monaxons would be comparable to a certain extent with the derelict spicules described above. I regret that I am not able to bring forward concrete evidence for these conclusions.

VI. HISTOLOGICAL NOTES; THE EXCRETORY CELLS OF *LEUCOSOLENIA COMPLICATA*.

The dermal epithelium of *Leucosolenia* may vary greatly in form, as Urban (1905, p. 53, pl. vi, figs. 42—62) has pointed out for another species. Some cells are of the conventional flat type (figs. 43, 54—57), from which every gradation can be found to others which have the nucleus placed deeper and are more flask-shaped (figs. 38, 47). Near the point of attachment the epithelium becomes of a pronounced columnar type (fig. 45). The species of *Leucosolenia* are very slightly, if at all, contractile, and contrast sharply in this respect with the *Clathrinidæ*. Hence, in agreement with Urban, I do not think that the flask-form can be explained in this genus as the result of contractility. I am not inclined, however, to regard the dermal epithelium as generally glandular, though it is very probably so in the region of the point of attachment. The clear protoplasm, free from coarse granules, of the general dermal epithelium does not suggest secretory cells. I regard the polymorphic nature of the dermal epithelium as due to the fact that it is practically a layer of amœboid cells, which are continually immigrating into the interior to form spicules and returning to the surface again. The power of concerted contractility possessed by the dermal epithelium of *Clathrinidæ* has apparently not been acquired by the *Leucosoleniidæ*, which in many respects present more primitive characters. In the collar-cells, for instance, the nucleus is terminal, as in

the larva, while in the Clathrinidæ the nucleus migrates into a position near the base of the cell.

Bidder (1898, p. 73) has pointed out that in *Leucosoleniidæ* the pore-cells, or, as he terms them, pylocytes, are not on the surface as in the Clathrinidæ, but at the bottom of short canals lined by the dermal epithelium. I am able to confirm this statement. Fig. 72 shows four porocytes and their relation to the dermal epithelium, drawn from the gastral aspect, the spicules and other cells being omitted. The structure of each pore and its enclosing cell is exactly as in Clathrina.

The amœbocytes in *Leucosolenia* are not so distinct from the other tissue-cells in appearance as they are in Clathrina, but can be distinguished by their larger nucleus (Figs. 49, 71, *amc.*). I found in *Leucosolenia* the same minute wandering cells, very abundant in some places, that I described formerly in Clathrina (fig. 71, *amc.*¹).

In the great majority of works upon sponge-histology, it is customary to distinguish and to describe a class of connective-tissue cells, stellate or bipolar in form. Without wishing to make statements about other sponges, it is my firm opinion that no such cells occur in Ascons. In surface views of the body-wall it is easy to make out the various classes of cell-constituents, namely, dermal epithelial cells, porocytes, amœbocytes, scleroblasts attached to the spicules, and collar-cells, with the addition, in certain specimens, of generative cells. No separate connective-tissue cells are to be seen. In sections of the body-wall, however, the spicules are always more or less displaced (compare fig. 55), and their scleroblasts, left in position, give the impression of separate connective-tissue cells. If, however, the stellate cells, frequently described as being numerous, really occur, they should be visible in surface views, in which their absence is, in my opinion, a convincing proof that no such class of tissue-elements occur in these sponges.

I come now to a remarkable class of cells, which I discovered first when studying the gastral rays of the quadri-

radiates in sections. On many of the gastral rays I found, in addition to the two formative cells one or two conspicuous rounded cells (figs. 64—67, *ex. c.*), packed full of coarse granules which take the carmine-stain more or less distinctly. In young gastral rays these cells were absent, and they were frequently absent also in full-sized rays; when present there were usually two, but in sections one of them may, of course, be cut away. The most striking peculiarity of these cells is that their coarse granules are always arranged in a layer at the surface of the cell, and in many cases appear to be in the act of being cast off from the cell (fig. 64—67, 69). The appearances suggest strongly, in short, that the coarse granules represent some kind of excretory material which the cells are producing and throwing off.

After finding these cells on the gastral rays I searched for them in other places, and soon discovered that they were fairly common among the collar-cells, from which they could easily be distinguished by the characters of their protoplasm (figs. 43, 53, 57, *ex. c.*). I also found them frequently in the oscular rim, at or close to the uppermost limit of the gastral collared epithelium (figs. 56, 57). Their presence in the oscular rim shows that they must be derived from the in-turned layer of dermal epithelium in this region, and that consequently they are cells of the same class as the porocytes, which they resemble in their characters. I take this opportunity of remarking that similar cells occur abundantly between the collar-cells of *Ascandra falcata*, projecting into the gastral cavity between the collar-cells, and that in *Clathrina coriacea* similar cells form the endogastral network described by me formerly (1899, p. 123, figs. 42 *c* and 46; 1900, p. 48).

As regards the existence of the excretory cells, as I believe them to be, upon the gastral rays, I was unable to decide to my own satisfaction whether they are formed where they are found, from the actinoblasts themselves, or whether they migrate on to the rays from their position between the collar-cells. If I am correct in regarding both the excretory

cells and the actinoblasts as derived from the porocyte-layer, then it is not impossible that the excretory cells might arise from the actinoblasts in some cases. Certain appearances suggest that the actinoblasts first become very granular and excretory in nature (fig. 59), and then give rise to separate excretory cells (fig. 69). On the other hand, I have frequently found the excretory cells near the base of young gastral rays, in a situation that suggests the possibility that they might migrate on to the rays (figs. 62, 63). I am obliged to leave this point undecided, and may remark that it would be better to study the question in surface-views of the inner aspect of the sponge-wall (as I had hoped to do) than in sections, since by the latter method it is never possible to be certain how much has been cut away in the preparation. Hence negative evidence is indecisive when derived from the study of sections.

With regard to the function of these cells, I may recall, in this connection the fact that Bidder (1892) regarded certain cells in *Clathrina clathrus* as excretory in function, basing this conclusion upon the reactions of these cells to stains. Bidder termed the cells in question Metschnikoff's cells. In my memoir on the *Clathrinidæ* (1898) I pointed out that the Metschnikoff's cells were contracted porocytes, and argued (l. c., p. 527) against the probability of the porocytes being excretory on the ground that any excretions produced by them would be carried into the sponge by the water-currents, whereas excretions are usually produced in situations where they are carried out of the body. I may point out, however, that the cells which I am describing in *Leucosolenia*, and which are also of the nature of porocytes, would be very favourably situated for exercising an excretory function, especially when they occur in the oscular rim or on the gastral rays of the quadriradiates. The cells of sponges, and especially of Ascons, are extremely generalised in function, and capable of exerting at different times activities which in other Metazoa are exerted by different cells, specialised each in a particular direction. Thus in

Clathrinidæ we see the contractile cells of the dermal epithelium migrating into the interior to become scleroblasts, and probably returning again to the epithelium when this function is discharged. In the same way it is not difficult to imagine a cell which under certain circumstances becomes a pore-cell and under others becomes a gastral actinoblast, may under yet other conditions take on an excretory function. If Bidder's term "Metschnikoff's cells" is to be used at all, I should suggest that it be used for cells such as I have described here, namely, cells of excretory function derived from the porocyte-layer, that is to say, from that part of the dermal epithelium which lines the oscular rim and furnishes the pore-cells and the gastral actinoblasts. And I may point out, that at a certain period in the development, or at any time during life in many Clathrinidæ, when they are contracted to a certain point, the porocyte-layer forms the innermost lining of the gastral cavity, excluding even the collar-cells from it (1900, figs. 58, 4, and 42, F).

The collar-cells of *Leucosolenia complicata* are shown in figs. 37 and 53; in other figures they are represented in outline. They are more or less flask-shaped, with the oval or pear-shaped nucleus at the upper extremity, close below the collar. The flagellum can be traced down to the nucleus. The collar is long and cylindrical; its free rim is difficult to make out. About half-way up the collar shows a hoop-like thickening. The cytoplasm is clear and finely granular, occasionally with a few coarser refringent granules and usually distinctly vacuolated. In sections it is common to find a collar-cell cut in such a way that only the base is shown. I have drawn one cut in this way in fig. 56 (*c. c.*) in order to show the difference between it and an excretory cell (*ex. c.*).

A cytological study of the collar-cell, its nucleus, and the mode of division would be of great interest. Unfortunately the osmic-picrocarmine method used by me, though very good for cytoplasmic details, gives very poor results for nuclear

structure. From appearances such as are figured in fig. 37, and which are frequently met with, the collar-cells of *Leucosolenia* would appear to divide longitudinally.

VII. AN ABNORMAL TRIRADIATE.

Fig. 101 on Pl. 20 represents part of an abnormal triradiate system of *Clathrina coriacea* with the cells upon it. This drawing was made by me more than ten years ago, but I have always kept it back in the hopes of finding other abnormal forms, and devoting a special memoir to them. As will be seen, one ray of the triradiate has two branches symmetrically placed on each side of the main shaft, each branch with a cell similar in appearance to a "thickener" attached to it.

From a single observation of this kind it is difficult and unsafe to draw conclusions or to attempt to reconstruct the course of events. Did the founder and thickener go off in different directions? Or, after the founder had gone off, did the thickener divide into two and so produce two daughter rays? It is unfortunate that the spicule, having reached the limit, apparently, of its growth, does not furnish any answer to these queries. Two things may be noted, however; one is that the three principal rays of the spicule are unusually stout and large, indicating great secretive activity on the part of the formative cells; the other point to be noted is that the two branch-rays are set on to the main ray at the same regular angle of 120° , which characterises the junction of the three principal rays.

VIII. SOME OBSERVATIONS OF THE DISPOSITION OF THE TRIRADIATE SYSTEMS IN *LEUCOSOLENIA LIEBERKÜHNII*.

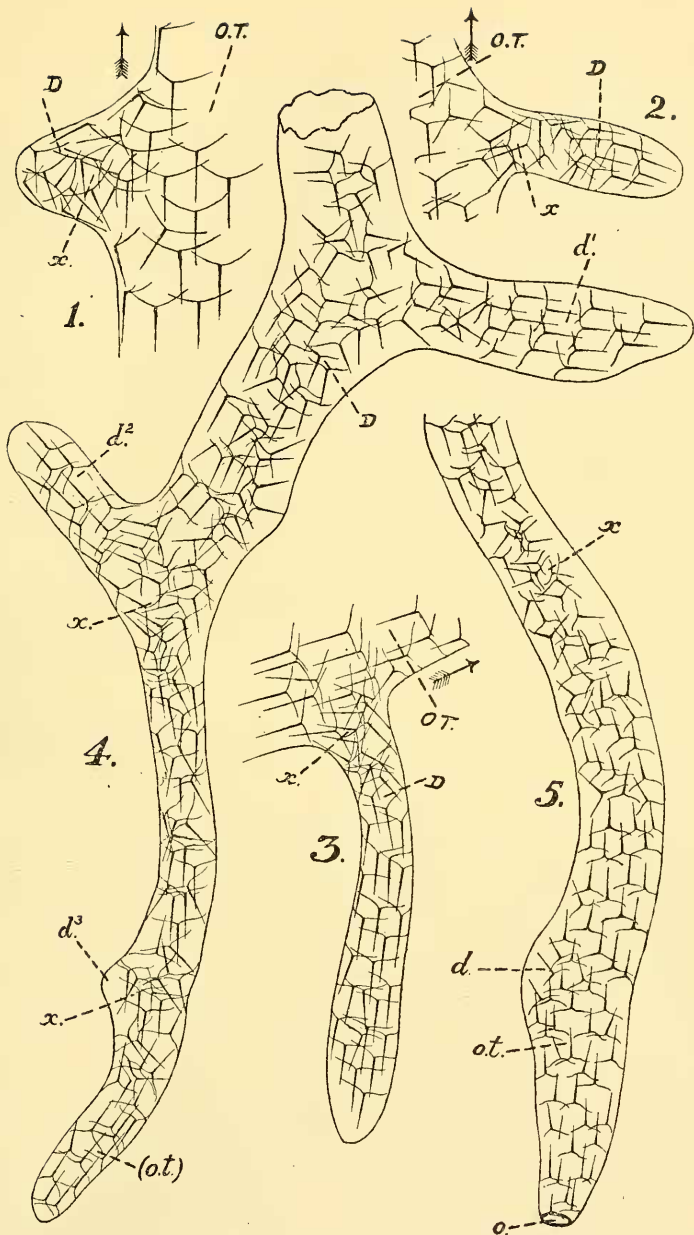
The observations to be recorded were made by me during my tenure of the Oxford Table in the Zoological Station at Naples during the year 1891-92. *Leucosolenia lieberkühnii* occurred then in the greatest abundance on the

bottom of a training ship moored permanently in the Porto Militare, growing amidst a thick crust of worm-tubes, Ascidians, sponges of various kinds, and other organisms. The colonies of *Leucosolenia* (Pl. 21, figs. 104—107) frequently attained a very large size, and were easily obtained from a boat by scraping the crust from the ship's bottom; during which occupation the too enthusiastic naturalist might sometimes receive on his head objects or liquids of a peculiar, and even offensive, nature from the port-holes of the vessel.

I made at that time a fairly complete study of the skeleton of this species of *Leucosolenia*, and found many errors in the published accounts of the spiculation. I will only refer at present to the peculiar slender monaxon spicules, shaped more or less like walking-sticks (Pl. 21, figs. 120—122), which I have found always present, though previously undescribed, in this species, not only in Neapolitan specimens but also in those from Banyuls, and other parts. I regard these spicules as most diagnostic of this species.

The triradiate systems (Pl. 21, figs. 109—114), both those with and those without an additional gastral ray, are, like those of other species of *Leucosolenia*, distinctly sagittal, having a straight unpaired "posterior" ray and two curved "lateral" rays. The "anterior" angle, between the paired rays, is greater than 120° ; the lateral paired angles are less than 120° . The unpaired ray is sometimes shorter, more often longer, than the paired rays.

In the accompanying text-figure I have represented the disposition of the triradiates in different parts of the sponges. I wish to state that, as regards form, the individual triradiates are represented diagrammatically in the text-figure without attempting to reproduce the exact form of the spicules; on the other hand, as regards position of the spicules, the figure claims to be exact. My method was to mount pieces of the sponge unstained in Canada balsam, and to draw with the camera lucida all the triradiates seen in the body-wall on the upper side of the tubes. Owing to the thinness of the body-wall the refringent spicules can be made



Leucosolenia lieberkühnii.—1. Portion of the oscular tube with a young diverticulum. 2 and 3. The same, with older diverticula. 4. Diverticulum bearing secondary diverticula. 5. A newly formed (secondary) oscular tube. *O.T.* Primary oscular tube. *o.t.* Secondary oscular tube. (*o.t.*). Ditto, in which the osculum is not yet formed. *O.* Osculum. *D.* Primary diverticula. *d, d', d², d³.* Secondary diverticula. *x.* Spots where the spicule-arrangement is confused. The arrows in 1, 2, and 3 point in the direction of the osculum.

out very easily in the transparent preparations. As the tubes are rounded, the spicules placed more towards the side are, of course, somewhat foreshortened. At first I used to draw the whole spicule, but I found it just as useful, and much less laborious, to draw only the straight posterior rays with the camera lucida, and to add the lateral rays freehand. It is for this reason that I state that the precise form of a given spicule is not to be considered as accurately represented in the figures. In fig. 108, Pl. 21, I have carefully drawn with a camera lucida the arrangement and form of the triradiate systems in one of the diverticula.

The sponge has erect oscular tubes, often of great size (Pl. 21, figs. 105, 107), from which grow out very numerous diverticula, ramifying in their turn (text-fig. 4). The diverticula are at first, and for a long time, blind at their ends (text-figs. 1—4), but become sooner or later perforated at their blind extremities to form oscula (text-fig. 5, *o.*), thus producing smaller oscular tubes which may be conveniently distinguished as secondary from the large erect primary oscular tubes.

Beginning the description with the primary oscular tube, a very uniform and regular arrangement of the triradiate systems is found (text-figs. 1, 2, and 3, *O. T.*; the arrows point in the direction of the oscular opening). Each triradiate is disposed with its straight unpaired ray pointing away from the osculum, and its two lateral rays extending in a direction as near to the horizontal as their form permits, that is to say, with their points slanting slightly upwards. In those systems which develop a fourth or gastral ray, and so become quadriradiates, it follows that the gastral ray curves forward to point towards the oscular opening, that is to say, that the gastral rays bend over and point in the direction of the water current, which flows up the oscular tube towards the terminal aperture. The suggestion that the gastral rays owe their curvature to the influence upon them of the water-current during development is an obvious one; we shall return to this point later.

From the oscular tubes arise, as already stated, numerous diverticula (*D.*), each an outgrowth of the body-wall pushed out, so to speak, like a glove-finger. Three stages are seen in text-figs. 1, 2, and 3. The triradiates formed in these diverticula take on from the first a different arrangement from those in the erect oscular tubes. At first the spicules are quite confused and irregular in arrangement (*x.*) As the diverticulum continues to grow, however, order emerges from chaos, and the triradiates are found uniformly disposed (text-figs. 2, 3, and Pl. 21, fig. 108). Each triradiate has its unpaired ray pointing towards the blind end of the diverticulum, but at the point of origin of each diverticulum from the oscular tube, there can be seen the region (*x.*) in which the chaotic and irregular arrangement of the triradiates is retained without any "righting" of the spicules having taken place. The arrangement of the triradiates in the diverticula of *Leucosolenia* was first pointed out by Bidder (1891, p. 627).

The diverticula are, of course, lined by collar-cells and perforated by pores, like the wall of the oscular tube, and it cannot be doubted that the water-current which flows in through the pores must pass along the diverticula in the direction from the apex to the base, finally debouching through the main aperture¹ into the oscular tube and joining the current in the latter. Hence it follows that the arrangement of the spicules in the diverticula is the same, in relation to the water-currents, as it is in the oscular tubes, with the exception of the basal region (*x.*). If the hypothesis that the gastral rays are bent by the current applies in the oscular tubes, it also applies in the diverticula.

The primary diverticula thrown out from the oscular tubes grow in length and produce secondary diverticula from their sides (text-fig. 4, *D*, *d*¹, *d*², *d*³). The formation of a second-

¹ Woodland in his comparison of the diverticulum to a "water cushion" (1905, p. 266) seems to have forgotten their aperture into the oscular tube. To my mind they can no more be compared to water-cushions than can the oscular tube itself.

dary diverticulum recapitulates exactly the events already described in the formation of a primary one; first, a chaotic arrangement of the triradiates (*x.*), then a quite regular disposition with the unpaired rays pointing to the blind apex.

When the diverticula have grown to a certain length, however, a remarkable change takes place in the arrangement of the spicules. In diverticula which still end blindly at the apex (text-fig. 4) it is seen that a short way from the apex the spicules become confused in arrangement and that from chaos an orderly arrangement again emerges (*o. t.*), in which, however, the disposition of the triradiates is exactly the reverse of what previously obtained; the unpaired ray points away from the blind apex of the diverticulum instead of towards it. As it cannot be supposed that the direction of the water-currents is changed so long as the diverticula end blindly, it follows that the gastral rays now point against the current, instead of bending with it.

The significance of the reversed arrangement of the triradiate becomes obvious when it is found that in other diverticula of about the same size or larger (text-fig. 5) the blind extremity becomes perforated by an aperture (*o.*) and gives rise to a secondary oscular tube. When this has taken place, the reversed arrangement of the triradiates is that which is proper to an oscular tube, and in the normal relation to the water-currents. Woodland (1905, p. 268) states that as soon as an osculum is formed "the young triradiates immediately assume the oscular arrangement," a process of rearrangement which he explains on his theory that the triradiates are "righted" by incident stresses and strains. He has entirely overlooked the fact that the triradiates can take on the oscular arrangements before ever an osculum exists; hence his whole argument falls to the ground. Whatever explanation may be given as to the causes which have operated in phylogeny in producing definite spicular arrangements, it is clear from the facts adduced that, in ontogeny, the spicules may take on a special arrangement before the conditions exist, to which their arrangement is adapted.

I may say at this point a few words about Woodland's theory of the "righting" of the triradiate systems. He states that even in the oscular tube the young triradiates of *Sycons* have an irregular orientation, which is later corrected by the action of direct stresses and strains on the wall of the sponge (1905, p. 265, footnote; 1907 [2], p. 76). His observations do not agree with mine on various species of *Leucosolenia*. Both in the oscular tubes and diverticula, I have always found the youngest triradiates to have from the first the orientation normal to the region in which they are found. The best way to see this is to take pieces of sponges preserved in alcohol, mount them stained or unstained in Canada balsam, and examine them by polarized light with crossed prisms; all the spicules appear brilliantly illuminated and the young stages are clearly seen. I must express my entire scepticism with regard to Woodland's theory of "righting." I believe the youngest spicules take from the first the position they have when full grown. That, at least, is my impression from the oscular tubes of *Ascons*. I suggest that the irregular orientation of the young triradiates in oscular tubes of *Sycons* is related to the numerous, closely set diverticula (radial tubes), continually being formed, in which a special arrangement of the spicules prevails, just as in *Leucosolenia*; and that it is comparable to the regions (*x*) where in *Leucosolenia* also the orientation of the spicules is chaotic, in relation to beginning, or even to future, evagination of the body-wall. These chaotic regions might be expected to be far more numerous in a growing *Sycon* than in a *Leucosolenia*. Compare the long, smooth oscular tubes of *Leucosolenia* (Pl. 21, figs. 104—107) with the young *Sycon* depicted by Maas (1900 [2], pl. x, fig. 7); his figure recalls the classical *Diana* of Ephesus.

Woodland gives a figure (1905, p. 271, text-fig. 10) to illustrate his theory that the body-wall is pressed in between the arms of the triradiates, so that "a groove is formed in line with each ray of the spicule along which the apical cell must tend to travel." I can confirm the first part of these state-

ments to some extent; the full-grown triradiates, placed more towards the dermal surface, can be seen plainly to bulge out the dermal surface so as to produce depressed areas in the angles between the rays, in all Ascons (compare fig. 71 *b*). Unfortunately for Woodland's theory, however, this only applies to the full-grown spicules; the young triradiates develop far in, close to the gastral surface, where the superficial depressions do not reach, and where consequently no grooves can direct the apical cells in their untutored wanderings.¹

IX. GENERAL REMARKS ON THE FORMATION OF CALCAREOUS SPICULES.

A spicule may be defined briefly as a sclerite, or skeletal element, of intra-cellular origin. The chief interest which

¹ I must point out that Woodland has occasionally fallen into some errors of statement in relation to objects which he has not himself studied. In one place (1905, pp. 238, 239, foot-note) he speaks of tuning-fork spicules in the stalk of *Clathrina lacunosa*. It is hardly necessary for me to point out that no such spicules occur in this sponge; Woodland was apparently thinking of the elbowed monaxons of the stalk, spicules of a very different type. Tuning-fork spicules are characteristic of the extinct Pharetronidæ and of some recent heterocœlous genera, such as *Lelapia*, probably allied to the Pharetrones; they are not known in any Ascon; and their position in the body of those sponges which possess them does not at all confirm Woodland's explanation of their form and characters. In another place (l. c., p. 274) Woodland states that "in no calcareous sponges do there co-exist small monaxons . . . with triradiates," but that either small and large monaxons, or large alone, are found. Now, the common *Leucosolenia botryoides* of our coasts, the earliest described species of Ascon, always has triradiates in great abundance, some of them very thick and massive; but its monaxons are frequently so small that they have been completely overlooked by spongiologists of repute, and require high powers to find them (compare Minchin, 1905 [2], text-fig. 97, p. 388). [Since this was written Woodland has corrected his statements concerning *Clathrina lacunosa* (1908, p. 148, footnote).]

Lapses of this kind are like the proverbial flies in the ointment; they weaken the theoretical conclusions which they are adduced to support, and they are a joy to the hostile critic. Woodland has himself produced such an abundant crop of valuable data upon spicule-formation that it is to be regretted that he did not rely more upon his own material as a basis for his speculations.

attaches to the study of spicule-formation arises from the fact that in some cases, at least, we have before us, as it were, the meeting ground of inorganic and organic forces. In calcareous sponges the spicules are almost entirely composed of calcite, which is a definite crystalline substance, and hence subject to laws which result from its physical structure. On the other hand, the spicules arise in living cells, and the forms which they assume are undoubtedly correlated with the bionomics of the organism rather than with the physical properties of the skeletal material. The fitness of the triradiate spicules for supporting the body-wall is obvious to anyone acquainted with the structure and life conditions of the sponge. On the other hand, the monaxons may be reasonably supposed to form an efficient protection to the sponge as well as, probably, contributing something to its support; while the gastral rays, curving up towards the oscular opening, very probably tend to keep out parasites, as Dendy has suggested, and I am quite unable to agree with Woodland (1905, p. 239) that they are entirely functionless.

Maas (1900 [1]) and Weinschenk (1905)¹ regard the spicule as the result of two activities, one organic-cellular, determining the form as a whole, the other inorganic-crystalline, responsible for the molecular structure of the contents. That this is, in a general way, a correct statement of the facts of the case no one can doubt. The chief question is the exact limitation between the respective spheres of influence of the vital and physical forces, and, in particular, whether, and to what extent, the form of the spicule may be influenced by its physical properties, that is to say, its crystalline nature.

In discussing this question we may begin by distinguishing

¹ "In every instant of the formation of a calcareous spicule, the deposition of the organically secreted calcite on the parts already present takes place according to purely inorganic laws of crystallisation, but there must always be present an organic element, which in its turn hinders the external inorganic form-production, and subordinates the external form to the conditions of the organism."—Weinschenk (1905, p. 584).

between primary spicules, derived each from a single mother-cell, which may divide to form formative cells, and aggregate¹ spicules, derived originally from several mother-cells, and representing a fusion of two or more primary spicules ancestrally distinct from one another. Instances in the Leucosoleniidae of primary spicules are the monaxons, and of aggregate spicules, the triradiates and quadriradiates.

If we consider the monaxon spicules first it does not appear to me possible to correlate their form in any way with their physical, that is to say, their crystalline properties. To begin with, they always have unlike poles, which is a point of difference from any crystalline body, but which can be correlated with their situation in the body-wall, one end being embedded in the tissues, the other projecting free into the water. The small monaxons of *L. complicata* are straight, but in most cases the shaft is curved in such a way as to cause the free apex to project more vertically outwards into the water. The frequent presence of a distinct barb at the free distal end is a character which may be regarded as increasing the efficiency of the spicule as a protective weapon, and I would invite those who find such an explanation too teleological for their taste to put forward an alternative theory, and to explain the presence of the barb on mechanico-physical grounds.

If crystalline properties fail to explain the forms of the monaxons, we may inquire what other causes can have produced the results that are before us. Woodland (1905), if I understand him rightly, considers that the presence of two nuclei as two centres of cell-activity, between which the spicule arises, explains the monaxon form. But we do not find that all monaxon spicules arise between two nuclei. The monaxon spicules of calcareous sponges certainly appear to do so invariably, but those of siliceous sponges, according to the

¹ I prefer this term, suggested by Woodland (1907, p. 56), to the term secondary spicules used by me formerly (1906). Woodland uses the term "secondary" spicules for those which are partly formed by the action of adventitious cells, such as the Lithistid desmas.

unanimous testimony of all observers, including Woodland (1908), are produced, and built up entirely, each by a single cell. Woodland has also described typical monaxon spicules of certain molluscs as arising each in one cell (1907 [1]). In calcareous spicules we have some important analogies presented by the formation of the gastral rays of the quadri-radiates. In considering this point there are two possible views to take with regard to the gastral rays; first, that they are sclerites of independent origin, not homologous at all with the monaxon spicules, in which case they do not furnish any analogy that can be used in my argument; secondly, that they represent, as I believe, monaxon spicules formed primitively in the interior of the oscular rim, which have become fused to the underlying triradiates. It may be asked, on this view, why monaxons formed on the dermal side did not also become fused to the triradiates. It is not easy always to suggest explanations for events that have occurred, but it is often still more difficult to explain why things have not happened that might possibly have taken place, and it can only be supposed that it is a more suitable arrangement for the monaxons that project on the exterior of the body-wall to be easily detachable structures, which are abandoned by their formative cells when completed. Now, if the homology of the gastral rays with monaxons be accepted, it is important to note that the gastral rays sometimes have one nucleus on them (*Clathrina cerebrum*), sometimes two, sometimes, as in *Clathrina contorta*, even four. On the ground of this analogy, as well as on more general grounds, I am inclined to think that the primitive sclerites from which the monaxon spicules of *Calcarea* arose were not originally formed between two nuclei, but by a cell with a single nucleus; and that the presence of two nuclei is a feature which was developed in correlation with the elongated, needle-like form, rather than antecedent to it.¹

¹ Woodland explains away the difficulty, on his theory, of the single cell on the gastral ray by assuming a co-operation between the actinoblast of the gastral ray and the basal formative cells of the triradiate system.

If, however, we waive these objections and assume that it is a universal rule for calcareous monaxon spicules to arise between two nuclei, we are still, it seems to me, very far from explaining the forms these spicules possess. The twin centres of secretive activity would only furnish us with a more or less elongated, bi-polar, intra-cellular structure, a type of spicule, in fact, such as is commonly found in siliceous sponges. We should still require some other factors to explain the projection outwards from the body wall, the frequent curvature, and above all the barb. Woodland's theory supplies, as it seems to me, nothing more than the simple rod-like spicule, which would be required on any theory of evolution as the starting-point for further bionomic adaptation; the raw material, as it were, for the action of selection or of the natural method, whatever it may be, by which the structural characters of organisms are brought into harmony with their needs as determined by the conditions under which they live.

To sum up the conclusions which I have endeavoured to establish in the foregoing paragraphs, it seems to me that the forms of the monaxon spicules are in no way explicable by the physical properties of the material, and only to a very limited extent, if at all, as the direct mechanical outcome of the conditions under which they develop. The monaxon spicules owe the peculiarities of their form chiefly and, perhaps, entirely to their relations to the sponge-body, and are adapted to the needs of the organism.

We may consider now the aggregate spicules; these, in calcareous sponges, always take the form of triradiate systems, to which a fourth ray may or may not be added.¹ Here we have two main types to consider, the perfectly equiangular triradiate systems found in *Clathrinidæ*, and the alate or sagittal spicules, with one unpaired and two paired angles found in *Leucosoleniidæ*. It is sufficient

¹ As pointed out above, the large monaxon spicules of *Clathrinidæ* are probably to be regarded, in some cases at least, as aggregate spicules, perhaps derived from modification of triradiate systems.

for the present to confine the discussion to the spicules of Ascons.

In my former memoir (1898) I rejected the notion that physical factors, such as crystallisation, had anything to do with the forms of the triradiates, and considered their symmetry to be entirely the result of adaptation. I have been forced to reconsider my position in this matter. The regular and unfailing symmetry of the triradiates in the species of *Clathrina* is a very striking thing, and becomes more so when we take other facts into consideration. Von Ebner has shown that in Clathrinid triradiates the facial plane, i. e. the plane containing the apices of the three rays, is at right angles to the crystalline optic axis, so that the morphological axes of the three rays lie entirely in three planes of crystalline symmetry which contain the optic axis, and intersect at angles of 120° ; and, further, that the straight gastral rays grow up in the optic axis, with which their morphological axis coincides. As Bidder (1898) has pointed out, the minute spines on the gastral rays of *Clathrina cerebrum* also form angles of 120° with one another, and repeat the symmetry of the basal rays. In the abnormal spicule figured by me (fig. 101) the additional rays come off from the main shaft at the same unfailing angle. If this angle is the result of adaptation, as I formerly argued,¹ it must be supposed that, by the operation of this principle over a vast time, the cells have acquired a hereditary tendency always to attach one spicule-ray to another at a fixed, invariable angle. This becomes difficult to imagine, when we find, as is frequently the case, that the three rays of a triradiate may be very irregular in the earliest stages, when they are still more or less separate from one another, and that they only become completely symmetrical when fused together; an observation directly opposed to the theory of hereditary tendency, which, as it seems to me, cannot go further than

¹ Maas, from his embryological observations, has pointed out that the form of the triradiates is in no way correlated with the arrangement of the pores, at least in ontogeny (1900 [2], p. 227).

to bring about simply the apposition of three mother-cells, and hence to produce three rays. It seems impossible to explain either by heredity or adaptation the definite relations of the rays to crystalline symmetry; some other cause must be sought for the equiangular arrangement. Woodland has explained the equiangular condition as due to development of the spicule "under undisturbed conditions" (1905, p. 270), while the sagittal triradiates are supposed to be the result of development in disturbed conditions.¹ I do not think this explanation can be accepted. Why should the spicules of a specimen of *Clathrina coriacea*, which lives between tide-marks and is subject to the rush of the tide four times in every twenty-five hours, roughly speaking, be supposed to develop under undisturbed conditions? Woodland has, moreover, confused together triradiates which are sagittal by deviations in the angles, and spicules which are sagittal simply by elongation of one ray, while remaining perfectly equiangular, as in *Clathrina blanca* and *C. lacunosa*. I find myself unable to attribute the wonderfully regular symmetry of the angles of the triradiates of *Clathrinidæ* either to adaptation, as I did formerly, or to the conditions under which the spicule develops, as Woodland does. I can only refer the regularity of the angles between the rays to the operation of the physical laws resulting from the crystalline properties of the material, which so modifies the growth of the rays relatively one to another, that it causes the three constituent elements of the compound spicular system to meet at the definite angles which are so constant a feature of these structures.

Various objections naturally occur to this view, which I will discuss. The first is, how is the symmetry of the *Leucosolenia*-triradiate to be explained, where equal angles between the three rays are the greatest exception, if

¹ I do not feel confident that I have quite grasped Woodland's meaning; the general trend of his argument seems to be that in the reticulate *Clathrinidæ* disturbances are balanced and do not preponderate in any one direction, as they do in the more erect *Leucosoleniidæ*.

indeed they ever occur.¹ Here we must consider the remarkable facts discovered by von Ebner concerning the relations of the triradiate systems to crystalline symmetry.

Von Ebner divides the triradiate systems of calcareous sponges into three main classes, which he terms perregular, sagittal, and irregular respectively. In the perregular triradiates, exemplified by those of *Clathrinidæ*, the optic axis is vertical to the facial plane of the rays, and all three rays are optically and morphologically equivalent² and meet at equal angles. In the sagittal spicules, seen in *Leucosoleniidæ* and *Heterocœla*, the optic axis is never vertical to the facial plane, and there are always an unpaired and two paired rays; the morphological axis of the unpaired ray lies in a plane of crystalline symmetry which contains the optic axis and halves the angle between the two paired rays. An irregular triradiate is defined as one in which no ray can be found, the morphological axis of which defines a plane of crystalline symmetry containing the optic axis and halving the angle between the other two rays; but since no irregular spicules, in this sense, could be discovered, it is doubtful if they exist at all, and it is not necessary to consider them further. The sagittal triradiates were further divided by von Ebner into primary and secondary forms. In the primary sagittal triradiates the morphological axes of all three rays lie entirely in planes of crystalline symmetry

¹ As von Ebner has pointed out, the three rays of a triradiate system seldom lie in one plane, but form the edges of a shallow pyramid, of which the base lies towards the gastral, the apex towards the dermal, surface. Hence a careful distinction must be drawn between the actual angles at which the rays join, and the apparent angles which they present in the facial projection, that is to say, when a spicule is seen under the microscope with its dermal surface uppermost and the apices of its rays resting on the slide. In the case of certain triradiates of *Leuconia solida*, von Ebner has shown that a spicule which appears regularly equiangular when viewed in the facial projection, may be, in reality, markedly sagittal if its real angles be measured accurately.

² One ray, as in *Clathrina blanca* and *C. lacunosa*, may be longer than the other two, without disturbance of other conditions. Von Ebner does not appear to have examined triradiates of this type.

which contain the optic axis and intersect at equal angles ; so that the triradiates of this class, if projected in a transverse plane (that is to say, in a plane at right angles to the optic axis), appear as regular equiangular triradiates, whatever their actual form may be.¹ In the secondary triradiates, on the other hand, the projection in a transverse plane gives a figure in which the angle between the paired rays is greater than 120° , usually 150° — 180° , and the curvature of the paired rays is not confined to a plane of crystalline symmetry which contains the optic axis. The secondary sagittal triradiates may be derived from the primary type by supposing that the paired rays rotate symmetrically away from each other, until they may come finally to lie in the same straight line when projected in a transverse plane of crystalline symmetry. An extreme form of the secondary sagittal type is seen in the "pseudo-regular" triradiates of the gastral surface of *Sycortis quadrangulata*; spicules which appear morphologically to be regular equiangular triradiates, but which are entirely different crystallographically from the true regular forms, since one ray, the unpaired ray, has its morphological axis coincident with the optic axis, while its paired rays both lie in one and the same plane of crystalline symmetry passing through the optic axis.

From the discoveries of von Ebner it is seen that the primary sagittal triradiates of *Leucosoleniidae* and *Heterocœla* agree with the perregular triradiates of *Clathrinidae* in so far, that both types alike appear equiangular when projected in a plane lying at right angles to the crystalline optic axis. With regard to the secondary sagittal forms, it can also be stated that their morphological symmetry is in a definite and constant relation to their crystalline structure. Ebner's investigations establish, so far as they go, the following

¹ This remarkable fact was also discovered by Bidder (1898), who regarded it as a universal law for all triradiate systems; Bidder was not aware, apparently, of the existence of von Ebner's secondary sagittal forms. In my paper at the British Association at York (1906) I also overlooked von Ebner's statements, and attributed the discovery to Bidder.

generalisation:—In all triradiate systems of calcareous sponges, whatever their form, there is one ray, the morphological axis of which lies entirely in a principal plane of crystalline symmetry, that is to say, a plane which includes the optic axis, and which also bisects the angle between the other two rays. Moreover, the ray which defines this plane of symmetry is the posterior ray, that is to say, the ray which, in the primitive Olynthus, points in the opposite direction to the oscular aperture, and therefore occupies, primitively at least, a definite position in the sponge-body. Thus, in the primitive orientation of the triradiate systems, such as is found to persist in the oscular tubes of Ascons, probably also in the oscular rim of any calcareous sponge, derived directly from the arrangement presented invariably by the embryonic Olynthus-form, the plane of crystalline symmetry defined by the posterior ray would also halve more or less accurately the entire sponge-body, since it would pass through the morphological axis of the body. The triradiate systems of the *Calcarea* exhibit a plan of crystalline symmetry which is in relation, not only with the morphological symmetry of the spicules themselves, but also with that of the sponge-organism. I think it may be reasonably inferred that this striking fact must be explained by physical peculiarities of the material, since it is a character of the spicules which cannot possibly have any biological or functional significance.

Maas (1904 [2], etc.) has advanced, as a proof that the form of the spicules is determined entirely by the organism in which they develop, the observation that in calcareous sponges grown in water deprived of CaCO_3 , the sclerites, though not containing calcite, still show the triradiate form. I do not gather, however, from Maas's memoirs, that these organic triradiate spicules have a regular symmetry, but simply that they consist of three rays joined together. As I have pointed out above, the operation of a hereditary tendency is an adequate explanation up to this point, and, in my opinion, no further.

To sum up briefly my conclusions with reference to the spicules of calcareous sponges; it is my opinion that the forms of primary spicules are determined solely by their relation to the organism and in no way by their crystalline structure, but that when primary spicules are joined together to form secondary systems, crystallisation may be a condition determining the angles at which they join. So long as the optic axis is vertical to the facial plane of the rays, the angles between the axes of the rays can only be 120° in the facial projection; variations in the angles first become possible by the rays becoming, as it were, displaced from their primitive relations to the planes of crystalline symmetry.

The conclusions reached in the foregoing paragraph may perhaps, as I have argued elsewhere (1905 [1]), be applied also to the second of the three principal stems of the sponge-phyllum, the Hexactinellida or Triaxonia; but in this case we must be more cautious, since there is no proof that in this group the spicules are crystalline in nature. The most striking feature of Hexactinellid organisation is the constancy with which the rays meet at right angles; and even when this peculiarity is masked by curvature or reduction of the rays, it is still shown clearly by the axial thread, as in the beautiful examples of the monaxons showing the "axial cross." Schulze (1887, pp. 501—504) first tried to give an explanation for the symmetry of the Hexactinellid spicule by ascribing it to an adaptation to the structure of the soft parts of the sponge, the triaxon spicule being shown to fit in perfectly between the thimble-shaped chambers suspended in the thick wall. As I have pointed out, it is highly probable that spicules were formed before chambers in the evolution of Hexactinellid sponges, in which case the form of the spicules could not have been determined by the arrangement of the chambers; and even if we assume that chambers were present before spicules, we do not get an adequate explanation of the triaxon form, since a layer of chambers, if disposed in the manner most economical of space, would tend to take on a honeycomb-like arrangement,

leaving interspaces to which an equiangular triradiate, not a cruciform spicule, would be the natural adaptation. Hence I consider that the constant rectangular junctions of the rays of Hexactinellid sponges cannot be regarded as an adaptation, but as an inherent peculiarity of the spicule itself, probably determined by the physical, if not the crystalline, properties of the material.

Strongly contrasting with the Hexactinellid spicules are those in the third sponge-stem, the heterogeneous assemblage united under the comprehensive name Demospongiæ by Sollas. Here the primitive spicule is apparently a tetraxon, or possibly an aster, of which the tetraxon is but one modification.¹ From this arises every possible type of form. Dendy has recently² constructed a phylogeny of the spicules of Demospongiæ. The only point which it is necessary for me to dwell upon here is, that in the Demospongiæ the angles at which the spicule-rays meet are infinitely variable, and show not the slightest tendency to be constant in any way. It is sufficient to mention the modifications of the triæne, distinguished by a formidable nomenclature (prottriænes, orthotriænes, anatriænes, etc.). It is evident that whatever may be the case in *Calcarea* and *Hexactinellida*, the physical nature of the spicule material in Demospongiæ offers no obstacle to the indefinite variability of the spicules. I see nothing against accepting Schulze's theory (1887, p. 503) that the primitive tetraxon of Demospongiæ arose as an adaptation to the form of the interspaces between numerous closely-packed spherical chambers. Comparison of existing forms, such as *Plakina* and *Oscarella*, make it highly probable that in this stem forms with chambers preceded forms with spicules in evolution.

It may be a stumbling-block to many that in *Calcarea* and *Hexactinellida* physical conditions should be regarded as a

¹ In this connection the statement of Maas (1900 [3]), that the asters of *Tethya* arise by fusion of separate tetraxons, must not be overlooked; but compare Woodland (1908).

² 'British Association Reports,' York, 1906.

factor in controlling the production of forms of spicules, while in Demospongiæ adaptive influences are allowed exclusive sway. It is a natural tendency of the mind to seek for uniform explanations in parallel cases. But if we accept Schulze's three main stems, or rather branches, of sponge-ancestry, it is probable, indeed, almost certain, that in each stem the spicular skeleton was acquired independently, and might therefore have been subject to different controlling influences in each case.

With regard to the evolution of the spicules within the group of the calcareous sponges I have but little to add to the conclusions I put forth in 1898 (p. 568). I then regarded the triradiate system as a fusion of three primitively separate monaxon sclerites, and I am more than ever convinced of this, now that I have found that the primary monaxons develop in a manner perfectly similar in every way to a single ray of a triradiate. I regarded, and still regard, the ancestral form of spicule in *Calcarea* as "a simple monaxon placed tangentially, and completely embedded, in the body-wall," and I believe that the typical monaxon of *Calcarea*, as now occurring, for instance, in *Leucosolenia*, arose from the primitive type by a process of accretion at one end, causing the older portion of the spicule to protrude from the surface of the body as the distal projecting extremity. The triradiates, on the other hand, arose by fusion of three primitive monaxons; and as I have pointed out above, the fusion to form triradiates took place by that extremity of the sclerite which, in existing monaxons, projects from the surface of the body.

From von Ebner's results, with regard to the regular triradiates, I think we may add a further conclusion, namely, that the primitive, tangential monaxon had its crystalline optic axis at right angles to its morphological longitudinal axis; in fact, that the optic axis of the ancestral monaxon sclerite was vertical to the body-wall, that is to say, radial to the longitudinal axis of the primitive *Olynthus*. The projecting monaxons of existing *Calcarea* have, as von Ebner has shown, the morphological axis inclined to the optic axis,

the inclination being greater or less in different parts of the spicule, according to the curvature, which is always in a plane containing the optic axis; and it is a striking fact that at the distal extremity of the spicule the optic axis is at right angles to the morphological axis. In other words, the oldest part of the monaxon, the part formed when the spicule lies entirely in the body-wall, has its optic axis orientated in the manner which was, on my view, the primitive and ancestral one for the spicules of calcareous sponges.

BIBLIOGRAPHY.

1891. BIDDER, G.—Review of Dendy, "A Monograph of the Victorian Sponges," 'Quart. Journ. Micr. Sci.' (N.S.), vol. 32, pp. 625—631.
1892. ——— "Note on Excretion in Sponges," 'Proc. Roy. Soc.,' li, pp. 474—484, 4 text-figs.
1898. ——— "The Skeleton and Classification of Calcareous Sponges," 'Proc. Roy. Soc.,' lxiv, pp. 61—76, 10 text-figs.
1887. EBNER, V, v.—"Ueber den feineren Bau der Skelettheile der Kalkschwämme nebst Bemerkungen über Kalkskelete überhaupt," 'Sitzber. k. Akad. Wiss. Wien,' 1 Abth., xcv Bd., pp. 55—149, pls. i—iv.
1872. HAECKEL, E.—'Die Kalkschwämme,' 3 vols. (Berlin, 1872).
- 1900 (1). MAAS, O.—"Ueber die sogen. Biokrystalle und die Skeletbildungen niederer Thiere," 'SB. Ges. Morph. Physiol. München,' 1900, 1, pp. 42—45.
- 1900 (2). ——— "Die Weiterentwicklung der Syconen nach der Metamorphose," 'Zeitschr. f. wiss. Zool.,' lxxvii, pp. 215—240, pls. ix—xii.
- 1900 (3). ——— "Ueber Entstehung und Wachsthum der Kieselgebilde bei Spongien," 'SB. math.-phys. Cl. Ak. Wiss. München,' xxx, pp. 553—569, pl. v.
- 1904 (1). ——— "Ueber die Wirkung der Kalkentziehung auf die Entwicklung der Kalkschwämme," 'SB. Ges. Morph. Physiol. München,' 1904, 1, 18 pp, 9 text-figs.
- 1904 (2). ——— "Ueber den Aufbau des Kalkskeletts der Spongien in normalem und in CaCO_3 freiem Seewasser," 'Verh. Deutsch. Zool. Ges.,' 1904, pp. 190—199.

1906. MAAS, O.—“Ueber die Einwirkung karbonatfreier und kalkfreier Salzlösungen auf erwachsene Kalkschwämme und auf Entwicklungsstadien derselben,” ‘Arch. Entwicklungsmech.,’ xxii, pp. 581—599.
1896. MINCHIN, E. A.—“Suggestions for a Natural Classification of the Asconidæ,” ‘Ann. Mag. Nat. Hist.’ (6), xviii, pp. 349—362.
1898. ——— “Materials for a Monograph of the Ascons. I. On the Origin and Growth of the Triradiate and Quadriradiate Spicules in the Family Clathrinidæ,” ‘Quart. Journ. Micr. Sci.’ (N.S.), vol. 40, pp. 469—587, pls. 38—42.
1899. ——— “Éponges Calcaires. La Clathrine coriace, *Clathrina coriacea* (Montagu),” ‘Zoologie Descriptive des Invertébrés,’ Paris, 1900, i, chap. v, pp. 107—147, figs. 35—52.
1900. ——— “Sponges,” in Lankester, E. R., ‘A Treatise on Zoology,’ part ii, chapter iii, 178 pp., 97 text-figs.
- 1905 (1). ——— “A Speculation on the Phylogeny of Hexactinellid Sponges,” ‘Zool. Anzeiger,’ xxviii, pp. 439—448, 2 text-figs.
- 1905 (2). ——— “The Characters and Synonymy of the British Species of Sponges of the Genus *Leucosolenia*,” ‘Proc. Zool. Soc. London,’ 1904, ii, pp. 349—396, text-figs. 91—96.
- 1905 (3). ——— “On the Sponge *Leucosolenia contorta*, Bowerbank, *Ascandra contorta*, Haeckel, and *Ascetta spinosa*, Lendenfeld,” ‘Proc. Zool. Soc. London,’ 1905, ii, pp. 3—20, pl. i, and 6 text-figs.
1906. ——— “Spicule Formation,” ‘Rep. Brit. Ass., York.’
1887. SCHULZE, F. E.—“The Hexactinellida,” “Challenger” Reps., Zool., xxi, 2 vols.
1905. URBAN, F.—“Kalifornische Kalkschwämme,” ‘Arch. f. Naturges.,’ 1906, i, 1, pp. 33—76, pls. vi—ix.
1905. WEINSCHENK, E.—“Ueber die Skeletteile der Kalkschwämme,” ‘Centrbl. Mineral. Geol. Palæontol.,’ No. 19, pp. 581—588.
1905. WOODLAND, W.—“Studies in Spicule Formation. I. The Development and Structure of the Spicules in Sycons; with Remarks on the Conformation, Modes of Disposition, and Evolution of Spicules in Calcareous Sponges generally,” ‘Quart. Journ. Micr. Sci. (N.S.),’ vol. 49, pp. 231—282, pls. 13—15, 11 text-figs.
- 1907 (1). ——— “Studies in Spicule Formation. VI. The Scleroblastic Development of the Spicules in some Mollusca and in one Genus of Colonial Ascidians,” ‘Quart. Journ. Micr. Sci.’ (N.S.), vol. 51, pp. 45—53, pl. 5, 1 text-fig.

- 1907 (2). WOODLAND, W.—“A Preliminary Consideration as to the Possible Factors concerned in the Production of the various Forms of Spicules,” ‘Quart. Journ. Micr. Sci.’ (N.S.), vol. 51, pp. 55—79.
- 1908 (1). ——— “Studies in Spicule Formation. VIII. Some Observations on the Scleroblastic Development of Hexactinellid and other Siliceous Sponge Spicules,” ‘Quart. Journ. Micr. Sci.’ (N.S.), vol. 52, pp. 139—157, pl. 7.

EXPLANATION OF PLATES 17—21,

Illustrating Mr. E. A. Minchin's paper on “Materials for a Monograph of the Ascons.”

On Plates 17—20 all the figures, except Fig. 101 on Plate 20, are drawn to a magnification of 1000 linear.

PLATE 17.

Figs. 1—8.—Development of the small straight monaxons of *Leucosolenia complicata*, as seen in surface views of the wall of the sponge.

FIG. 1.—Scleroblast with two nuclei and commencing separation of the two formative cells. No trace as yet of spicule-formation.

FIG. 2.—Similar stage, but with the first foundation of the spicule indicated as a delicate line, passing obliquely between the two nuclei.

FIG. 3.—The delicate line of the last stage has expanded into a clear, fusiform area, sharply limited from the surrounding cytoplasm. No trace as yet of mineral deposit.

FIG. 4.—Similar stage to the last; the clear space is larger, and running through part of it a delicate axial line could be made out.

FIG. 5.—Commencement of mineral spicular deposit within the clear space, which it far from fills.

FIG. 6.—A later stage; the “thickener” beginning to travel away from the projecting distal extremity of the spicule.

FIG. 7.—Spicule nearly fully formed; the thickener quite close to the founder.

FIG. 8.—Spicule complete. The founder apparently wandering off, the thickener at the proximal extremity.

FIGS. 9—18.—Development of the large curved monaxons of *L. complicata*, as seen in surface views of the wall of the sponge.

FIG. 9.—Lance-head between the two nuclei of the formative cells, the separation between which is as yet scarcely indicated.

FIG. 10.—Lance-head with the two formative cells at each extremity.

FIG. 11.—Similar stage; the lance-head much thicker; the shaft of the spicule growing rapidly in length.

FIG. 12.—Later stage; the thickener commencing to leave the completed lance-head.

FIG. 13.—The lance-head abandoned; the activity of the thickener shown by a distinct rim (*r.*) on the shaft.

FIG. 14.—Similar stage; the rim (*r.*) formed by the thickener very distinct. The founder in its progress has encountered a ray of a triradiate system (*r.t.s.*), producing a distinct kink in the spicule (*l.*).

FIG. 15.—Similar stage.

FIG. 16.—Similar stage in the variety of the large monaxons which is without a lance-head.

FIGS. 17, 18.—Spicules nearly fully formed, showing the two formative cells close together at the proximal extremity of the spicule.

FIGS. 19—29.—Development of the triradiates and quadriradiates of *L. complicata* as seen in surface views. The nuclei seen at a higher focus in the preparation are coloured more deeply than those seen at a lower focus.

FIGS. 19, 20.—Two sextetts in which the first trace of spicule-formation is seen in the form of irregular granules. In one instance the granules are in a distinct vacuole.

FIG. 21.—Sextett containing three small pieces of calcareous matter, one larger than the others, and evidently taking shape as the unpaired ray.

FIG. 22.—Septett containing a large and distinct unpaired ray and traces of one of the lateral rays and of the gastral ray.

FIG. 23.—Sextett with small triradiate, lying in a clear space limited by a distinct sheath.

FIG. 24.—Small quadriradiate with six basal formative cells and gastral actinoblast with two nuclei.

FIGS. 25, 26.—Small quadriradiates with still a single nucleus in the gastral actinoblast.

FIG. 27.—Small triradiate with six formative cells.

FIG. 28.—Older quadriradiate. The gastral actinoblast, with two nuclei, has a polygonal outline with incurved sides and sharp corners, caused by fitting in between the bases of the collar-cells.

FIG. 29.—A nearly full-grown quadriradiate with all six formative cells. Gastral actinoblast not drawn.

FIGS. 30—36.—Derelict triradiates and quadriradiates, some with a few cells still attached, others with none. All occurring close to the dermal surface.

FIGS. 37—41.—Histology and spicule-formation of *Leucosolenia complicata*, as seen in sections.

FIG. 37.—An ordinary collar-cell (on the left) and two sister-cells resulting from recent division. In the latter the collars are not yet formed, and the flagella are short, thickened at their base, and tapering to a point.

FIG. 38.—A cell of the dermal epithelium, mushroom-like type; the body of the cell, containing the nucleus, is placed far down the surface, and sends out delicate processes.

FIG. 39.—A cell of the dermal epithelium in which the nucleus has recently divided.

FIG. 40.—Two sister-cells of the dermal epithelium resulting from recent division.

FIG. 41.—Cells of the dermal epithelium passing in to form a spicule (compare Figs. 1 and 2).

PLATE 18.

Histology and spicule-formation of *Leucosolenia complicata* as seen in sections (collar-cells drawn in outline, except in Fig. 53).

FIG. 42.—Section showing dermal epithelium and a young monaxon spicule (*m.sp.*) with its two scleroblasts. No nuclei of epithelial cells come into the section. The other parts of the section are not figured.

FIG. 43.—Section showing a young monaxon spicule (*m.sp.*) with its two formative cells (the distal end of the spicule broken off); two cells of the dermal epithelium (*d.ep.*); five collar-cells, and amongst them an excretory cell (*ex.c.*).

FIG. 44.—Section showing a monaxon spicule (*m.sp.*) at a late stage of growth (compare Fig. 7); the spicule is much broken in cutting. Two cells of the dermal epithelium and three collar-cells are also seen.

FIG. 45.—Portion of a section through the region where the sponge is attached to an alga (*A.*); the dermal epithelium (*d.ep.*) becomes at this point very columnar; the mesogloea is greatly thickened. *sel.t.* Scleroblast attached

to a fragment of a triradiate. *sel.m.* Two formative cells of a monaxon spicule, which has begun to be formed as a clear space (compare Figs. 2, 3, and 4 on Pl. 17). Four collar-cells are seen.

FIG. 46.—Section of the oscular rim, showing five cells of the dermal epithelium; in one of them a portion of the shaft of a monaxon spicule (*m.sp.*) is imbedded. The two uppermost collar-cells (*c.c.*) come into the section.

FIG. 47.—Portion of a section of the oscular rim, showing four cells of the dermal epithelium; in one of them is embedded a monaxon spicule, which is cut across.

FIG. 48.—Section of an early stage of one of the large, curved, barbed monaxons (compare Fig. 9 on Pl. 17). The section was thick; hence the spicule, which was placed obliquely in the section, is much foreshortened in the drawing.

FIG. 49.—Portion of a section showing two cells of the dermal epithelium (*d.ep.*); a wandering cell (*am.c.*); two cells of a sextett (*sel.*); and six collar-cells, in one of which only the base is seen in the section; besides fragments of spicules.

FIG. 50.—Portion of a thick section, showing a young quadriadiate; on the basal system five of the six formative cells are seen; the gastral ray (*g.r.*) projects between the collar-cells and bears an actinoblast with two nuclei (compare Figs. 24 and 28 on Pl. 17).

FIG. 51.—Section showing a very young quadriadiate; five of the formative cells of the basal system are seen, and the large, granular, gastral actinoblast (*g.act.*) with its single nucleus (compare Fig. 26 on Pl. 17).

FIG. 52.—Young quadriadiate, intermediate in size between those drawn in the two preceding figures. Four formative cells of the basal system are seen, and the gastral actinoblast has two nuclei.

FIG. 53.—Three collar-cells, drawn from a section, with a granular excretory cell (*ex.c.*) amongst them.

FIG. 54.—Section passing through a pore (*P.*). On the right of it are seen two dermal epithelial cells (*d.ep.*), a sextett (*sext.*), and a cell (*p.c.*) which may be either the principal portion of the porocyte, or a gastral actinoblast.

FIG. 55.—Section showing a young triradiate embedded in its sextett of formative cells (*sext.*).

FIG. 56.—Section through the lower part of the oscular rim. On the right an excretory cell (*ex.c.*) is seen amongst the collar-cells; one collar-cell (*c.c.*), of which only the base appears in the section, has been filled in, to show the difference between its cytoplasm and that of the excretory cell. On the left is seen the gastral ray (*g.r.*) of a quadriadiate; near it is a dermal epithelial cell, which sends out a prolongation ensheathing the gastral ray.

FIG. 57.—Section of the oscular rim; on the extreme right the first collar-cell is drawn in outline; on the inner (lower) side four cells of the dermal epithelium come into the section, but on the outer side none are cut through, since the section passes between them. *m.sp.* Menaxon spicule with two formative cells. *scl.* Scleroblast on the ray of a triradiate cut across. *ex.c.* Excretory cell.

FIG. 58.—Young triradiate from a section showing the unpaired ray in its full length, bearing the basal and apical formative cells (*b.f.c.*, *a.f.c.*). The two paired rays are cut off close to their origins, but show their basal formative cells.

FIG. 59.—Gastral ray of a quadriradiate, from a section showing the granular actinoblast with two nuclei.

PLATE 19.

Figs. 60—70.—Gastral rays of quadriradiates, from vertical sections of the body-wall of *Leucosolenia complicata*.

FIG. 60.—Young quadriradiate showing the unpaired basal ray, broken, with the two formative cells (*b.f.c.*, *a.f.c.*), and the gastral actinoblast (*g.act.*), with two nuclei.

FIG. 61.—Young quadriradiate showing two scleroblasts of the triradiate system and the gastral actinoblast (*g.act.*) with two nuclei, which have apparently originated by very recent division of the original nucleus.

FIG. 62.—Quadriradiate with the gastral ray (broken in two places) bearing the actinoblast with two nuclei, and close to the base, among the collar-cells, an excretory cell (*ex.c.*).

FIG. 63.—A quadriradiate, showing two formative cells of the basal system, and two nuclei on the gastral ray, near the base of which is seen an excretory cell (*ex.c.*), apparently connected with the gastral actinoblast by a protoplasmic process.

FIG. 64.—A gastral ray of a quadriradiate, showing the tip enveloped by the actinoblast, and with an excretory cell (*ex.c.*) attached, from which granules are being ejected. It was difficult to be quite certain if the actinoblast contained one or two nuclei; possibly one has been cut off.

FIG. 65.—Similar to the last; gastral actinoblast distinctly with two nuclei.

FIGS. 66, 67.—Full-grown gastral rays, each enveloped in an actinoblast bearing two nuclei (*n.act.*, *n.act.*), and carrying two excretory cells (*ex.c.*).

FIG. 68.—Portion of a section through the oscular rim; the first collar-cell is seen on the extreme right. *g.r.* A gastral ray covered partly by the dermal epithelium (*d.ep.*), but not bearing an actinoblast or excretory cell.

FIG. 69.—A gastral ray, of which the actinoblast is apparently giving rise to an excretory cell.

FIG. 70.—A gastral ray, enveloped in an actinoblast with two nuclei, but without any excretory cells.

FIG. 71.—A portion of the body-wall, seen from the gastral aspect, with the collar-cells brushed away; the figure shows, besides spicules and their scleroblasts and cells of the dermal epithelium, two pores (*P.*), a sextett (*sext.*), three ordinary amœbocytes (*amc.*), and numerous minute amœbocytes (*amc^l.*).

FIG. 71*a*, 71*b*.—Portions of sections showing the proximal ends of large monaxon spicules (*M.SP.*) bearing each two formative cells (*f.c.*, *th.c.*). In 71*b* are seen also two cells of the dermal epithelium.

FIG. 72.—Surface view of the body-wall seen from the gastral aspect; only the dermal epithelium (*d.ep.*) and the porocytes (*p.c.*) are drawn, to show the way in which the epithelium lines shallow depressions, at the bottom of which the pores are found.

PLATE 20.

Figs. 73—100.—Development of the spicules of *Leucosolenia variabilis*, as seen in surface views of the body-wall.

Figs. 73—83.—Development of the small monaxons.

Figs. 73—75.—Formation of the two scleroblasts by division of a cell of the dermal epithelium.

Figs. 76—78.—Youngest stages of the spicules; the lance-head embedded in the "thickener;" the shaft, as yet scarcely formed, is being laid down by the "founder."

Figs. 79—81.—The thickener leaves the fully formed lance-head and is travelling down the shaft, building it up to its full thickness; the founder prolongs the spicule at its proximal end.

Figs. 82, 83.—Monaxons nearly full-grown, with the formative cells close together at the proximal end of the shaft.

Figs. 84—86.—Three stages in the formation of medium-sized monaxons. Fig. 84 is a young stage, showing the lance-head still embedded in the thickener. Figs. 85 and 86 show spicules nearly full-grown, each with the formative cells close together at the proximal end of the shaft.

Figs. 87—89.—Final stages in the formation of the large-sized monaxons. In 87 and 88 the two formative cells are still present on the proximal end of the shaft, which tapers evenly to a sharp point. Fig. 89 is a spicule completely formed; the proximal extremity tapers abruptly and bears no formative cells.

Figs. 90—100.—Development of the triradiate and quadriradiate systems.

Figs. 90, 91.—Sextetts. In Fig. 91 the small size of the nuclei indicates recent origin by division from the three actinoblasts.

Figs. 92—95.—Four young quadriradiates, drawn from the dermal side; the spicules are slightly corroded by the glycerine. On each ray are seen the two formative cells, basal "thickener," and apical "founder." The gastral actinoblasts, being under the spicules, are not clearly seen, but can be made out to contain each a single nucleus. In Fig. 94 two cells of the overlying dermal epithelium (*d.ep.*) are drawn.

Figs. 96—100.—Rays of triradiate and quadriradiate systems, showing the migration of the "thickener" to the apex of the ray and the disappearance of the "founder." Figs. 98 and 100 are full-grown spicules, having the thickeners on the extreme points of the rays.

Fig. 101.—Abnormal triradiate of *Clathrina coriacea*. $\times 1250$ linear. One of the rays has branched into two, each branch bearing a scleroblast.

Figs. 102, 103.—Amœbæ found commonly on the exterior of *Leucosolenia variabilis*. $\times 1000$ linear.

PLATE 21.

Spicules, etc., of *Leucosolenia lieberkühnii* from Naples.

Figs. 104—107, natural size. Figs. 108—122, $\times 170$ linear.

Figs. 104—107.—Specimens of the sponge. Fig. 107 shows an unusually large oscular tube.

Fig. 108.—Distal end of a diverticulum; on the surface only the triradiate systems and pores are drawn; at the edge, in optical section, only the monaxons are shown.

Figs. 109—112.—Triradiates, isolated.

Figs. 113, 114.—Quadriradiates.

Figs. 115—119.—Ordinary monaxons.

Figs. 120—122.—"Walking-stick" monaxons. In Fig. 122 the distal extremity is shown more highly magnified.